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(57) Abstract

The present invention is based upon the discovery that the use of pyruvate in transplant solutions and the feeding of pyruvate and/or pyruvate derivatives to the organ recipients prior to and subsequent to transplantation, greatly enhances the survivability of the recipient and the viability of the transplanted organ. According to the present invention, providing pyruvate prior to the transplantation of an organ and/or subsequent to the transplantation of the organ, will significantly increase the likelihood of a successful transplantation. Further, the use of pyruvate-containing preservation, transplant, perfusion and pyruvate irrigation solutions will greatly enhance the maintenance of the organs prior to transplantation. The transplant and irrigation solutions of the present invention contain components that are known in the art and pyruvate at a concentration from 1 to 100 mM.

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TRANSPLANT SOLUTIONS CONTAINING PYRUVATE AND METHODS FOR TRANSPLANTATION

Technical Field

The present invention relates generally to methods of transplanting tissue and organs in mammals and to solutions useful in the transplant of tissues and organs which contain pyruvate and pyruvate derivatives.

Background Art

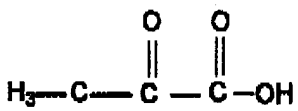
The transplantation of organs and/or tissue from a donor to a recipient has become almost routine in modern medicine. Modern preservation methodologies have provided for the maintenance of organs over relatively long periods of time prior to transplantation. Despite this, present preservation technology is limited and there is a need to improve the viability of the organs or tissue to be transplanted over longer periods of time. Improving techniques for transplantation would have far ranging implications for clinical application and basic research on organ transplantation.

The present invention is based upon the discovery that the use of pyruvate in transplant solutions (for preservation and irrigation during surgery) and the feeding of pyruvate and/or pyruvate derivatives to the organ/tissue recipient prior to and subsequent to transplantation, greatly enhances the survivability of the recipient and the viability of the transplanted organ/tissue.

As used herein and in the claims, the term "organ" is meant to mean a differentiated structure, such as the heart, kidney or liver, which consists of cells and tissues and performs some specific function in an organism. Also included in the definition of "organ" is the term "tissue"

which is an aggregation of cells more or less of similar morphology and functionality. The animal body is composed of four (4) primary tissues, namely, epithelium, connective tissue (including bone, cartilage and blood), muscle and nervous tissue.

As used herein and in the claims, the term "pyruvate" means any salt or esters of pyruvic acid. Pyruvic acid has the formula:



Pyruvic acid is a colorless liquid with an odor resembling that of acetic acid and has a melting point of 13°C. Pyruvic acid is an intermediate in the breakdown of sugars to alcohol by yeast. The mineral salts of pyruvic acid, such as sodium pyruvate or calcium pyruvate or mixtures thereof are useful in the present invention. Pyruvate precursors in the form of pyruvamides or pyruvyl-amino acids are also useful in the present invention. Pyruvyl-glycine is representative of the useful pyruvyl-amino acids.

Pyruvate has a number of useful applications in medicine. Pyruvate has been described for retarding fatty deposits in livers (U.S. Patent No. 4,158,057); for treating diabetes (U.S. Patent No. 4,874,790); for retarding weight gain (U.S. Patent Nos. 4,812,879, 4,548,937, and 4,351,835); to increase body protein concentrations in a mammal (U.S. Patent No. 4,415,576); for treating cardiac patients to increase the cardiac output without accompanying increase in cardiac oxygen demand (U.S. No. Patent 5,294,641); for extending athletic endurance (U.S. Patent No. 4,315,835); for retarding cholesterol increase (U.S. Patent No. 5,134,162); for inhibiting growth and spread of malignancy and retarding DNA breaks (U.S. Application Serial

No. 08/194,857 filed February 14, 1994); and for inhibiting generation of free radicals (U.S. Application Serial No. 08/286,946 filed August 8, 1994). All of these references are incorporated herein by reference.

Pyruvate in various forms has been proposed for enteral administration and for parenteral administration. Typically, pyruvates are available in the form of salts, for example, calcium pyruvate and sodium pyruvate. U.S. Patent Nos. 5,283,260 and 5,256,697 disclose uses for the pyruvyl-amino acids and methods for their production.

Pyruvate has been administered to mammals enterally or parenterally typically at superphysiological levels. The amount of pyruvate administered generally ranges from 1 to 20% of the mammal's caloric intake. For enteral administration, the pyruvate may be disbursed or dissolved in a beverage product or may be included in cookies, candies or other foods. Pyruvate may also be introduced as an aqueous solution parenterally.

According to the present invention, providing pyruvate to the donor animal prior to the transplantation of an organ and/or to the recipient subsequent to the transplantation of the organ, will significantly increase the likelihood of a successful transplantation. Further, the use of pyruvate-containing transplant solutions or pyruvate irrigation solutions will greatly enhance the maintenance of the organs prior to and during transplantation.

Transplantation is the removal of part of an organism and its replacement in the body of a different individual. The transplantation of cells, tissues or organs is an important procedure for treating a number of diseases and for studying a wide variety of problems. Transplantation of organs is a widely accepted medical therapy for diseases such as cardiac disease, liver disease and diabetes.

Transplantation involves two types of problems; those concerned directly with the act of transplantation itself and those which stem from the incompatibility of the donor organ to a genetically dissimilar recipient. An aspect of the present invention comprises administering pyruvate to the donor (if possible) and the recipient animal prior to and/or subsequent to organ transplant. The transplant solutions (for preservation and irrigation) containing pyruvate of the present invention also lessens the injury to the transplanted organ during periods of preservation and during the reperfusion event. Restoration of the flow of blood to a particular region of an animal (i.e., muscles or organs) is called reperfusion. One additional aspect of the present invention resides in the discovery that accepted solutions used to transport the donated organ which contain pyruvate produce an unexpected benefit in the preservation of the organ.

Disclosure of the Invention

There is disclosed a transplant solution comprising water, a buffer system and pyruvate. The concentration of the pyruvate can range from 1-100 mmol or mM (millimolar). More preferably, the concentration of the pyruvate can range from 1-25 mM. Preferably, the pyruvate is selected from the group consisting of sodium pyruvate, calcium pyruvate, potassium pyruvate and mixtures thereof. Further, the transplant solution of this invention may contain antibiotics, steroids, insulin, nucleotides, carbohydrates, peptides and other components that are currently accepted in transplant, irrigation and perfusion solutions.

There is also disclosed a method for the transplantation of an organ in a mammal, said method comprises the administration of pyruvate enterally or parenterally to the recipient mammal prior to and/or subsequent to the transplantation.

There is further disclosed a method for the transplantation of an organ in a mammal, said method comprises the perfusion of the organ with a transplant solution comprising water, a buffer system and pyruvate prior to the establishment of the blood flow from the recipient to the organ.

There is also disclosed a transplant solution comprising water, at least one steroid at a concentration of from 2-20 mg/L. at least one carbohydrate at a concentration of from 10 to 100 mM, at least one nucleoside at a concentration of 1-20 mM, at least one antibiotic at a concentration of 0.1 to 1.0 mg/L and pyruvate at a concentration of 1 to 100 mmol and wherein said transplant solution has an Osmolality of 250-375 mmos.

As used herein and in the claims, the term "pyruvate" means the mineral salts of pyruvic acid, for example the sodium, potassium or calcium salts, and pyruvate precursors in the form of pyruvamide or a pyruvyl-amino acid. Examples of the pyruvyl-amino acids useful in this invention are pyruvyl-glycine, pyruvyl-alanine, pyruvyl-leucine, pyruvyl valine, pyruvyl-isoleucine, pyruvyl-phenylalanine, pyruvyl-proline, pyruvyl-sarcosine and their amides and esters as well as their salts. The teachings of U.S. 5,256,697 which relate to methods of administering pyruvate and to methods of synthesizing pyruvate precursors is incorporated herein by reference.

As used herein and in the claims, the term "buffer system" means medically accepted salts of a weak acid in the presence of the free acid itself or the salts of a weak base in the presence of the free base itself. "Buffer solutions" which are aqueous solutions of "buffer systems" are known in the medical and biological arts. In medicine, it is desirable to prepare a solution of definite pH, made up in such a away that this pH alters only gradually with the addition of alkali or acid.

Those skilled in the medical will readily appreciate which buffer systems would be useful in the present invention.

As used herein and in the claims, the term "transplant solution" means an aqueous preservation solution that is used to store the organ to be transplanted, an irrigation solution which is used to flush a body part for removing a foreign object or medicating the body part, a perfusion solution for forcing through an organ especially by way of the blood vessels and an intravenous solution that can be used to administer the pyruvate to the organ recipient.

There is also disclosed a method for the transplantation of an organ in a mammal, said method comprising the administration of pyruvate to the recipient mammal prior to the transplantation of the organ. This invention also contemplates the administration of pyruvate to the recipient mammal subsequent to the transplantation of the organ. The method also contemplates the enteral and parenteral administration of the inventive transplant solution to the recipient mammal.

Detailed Description of the Invention

An accepted solution used for the transplantation of organs is the University of Wisconsin Transplant Solution. This solution consists of the following components at the recited concentrations:

K Lactobionate	100 mmol
KH2PO4	25 mmol
MgSO4	5 mmol
Raffinose	30 mmol
Adenosine	5 mmol
Glutathione	3 mmol
Insulin	100 U/L

Bactrim	0.5 ml/L
Dexamethasone	8 mg/L
Allopurinol	1 mmol
Hydroxyethiol-starch	50 g/L
Osmolality	320 mmos
pH	7.4
Na	30 mmol
K	120 mmol

The preservation or irrigation solution of the present invention can be the Wisconsin solution, which additionally contains pyruvate, at concentrations of from 1 to 25 mmol, more preferably from 2 to 15 mmol. More generally, the solutions according to this invention can utilize conventionally accepted components, such as energy sources (i.e., sugars and carbohydrates), antibiotics, anti-inflammatories, buffer systems, mineral salts and the like. An especially preferred solution of the invention comprises a standard Ringer's solution containing 1 to 25 mmol of sodium or calcium pyruvate. Ringer's solution is a physiological saline solution which is isotonic with the serum of blood and is used for perfusion and tissue culture experiments. One important aspect of the solution of this invention is that it be isotonic with the blood of the animal in which they will be used. Solutions having the same osmotic pressure are termed isotonic.

In one aspect of the present invention, pyruvate is administered to the transplant recipient prior to and subsequent to the transplant operation. The level of pyruvate can range from 1 to 20% of daily calories. Preferably, the pyruvate replaces a portion of the carbohydrates in the diet.

EXAMPLE I

Transplant Pancreatic Islet Cells

Short and long term preservation of pancreatic islet cells has been accomplished through low temperature cryopreservation. Utilizing current technologies, unacceptably low cell yields have limited the clinical transplantation of pancreatic islet cells to treat diabetes. One aspect of the present invention resides in the discovery that long term culture of pancreatic islet cells in a pyruvate-rich medium enhances the viability and function of human pancreatic islets.

Islets were isolated from human pancreata using the method described by Ricordi et al in Diabetes 37:413, 1988. After isolation, the islets were further purified by velocity sedimentation on a discontinuous Eurocollins ficoll density gradient. The cells were cultured at 37°C in 5% CO_2 in air either in a pyruvate supplemented medium at 4-15 mmol or in a standard culture medium. The standard culture medium was CMRL-1066 supplemented with 5-10% fetal calf serum. The standard culture medium CMRL-1066 has the following composition:

Component	mg/L
INORGANIC SALTS	
$CaCl_2$ (anhyd.)	200.00
KCl	400.00
Mg_3O_4 (anhyd.)	97.70
NaCl	6800.00
$NaHCO_3$	2200.00
$NaH_2PO_6 + H_2O$	140.00
OTHER COMPONENTS:	
Cacarboxylase	1.00
Coenzyme A	2.50
2-Deoxyadenosine	10.00
2-Deoxycyidine + HCl	10.00
2-Deoxyguanosine	10.00
Oiphosopropyridine Nucleotide	7.00
Flavin Adenine Dinucleotide	1.00
D- Glucose	1000.00
Glutathione (reduced)	10.00
5-Methyl-deoxycytidine	0.10
Phenol Red	20.00
Sodium Acetate + $3H_20$	83.00
Sodium Glucuronate + H_20	4.20
Thymidine	10.00
Triphosphopyridine Nucleotide	1.00
Tween 80	5.00
Uridine Triphosphate	1.00
AMINO ACIDS	
L-Alanine	25.00
L-Arginine HCl	70.00
L-Aspartic Acid	30.00
L-Cystine - 2HCl	26.00
L-Glutamic Acid	75.00
L-Glutamine	100.00
Glycine	50.00

Component	mg/L
AMINO ACID (cont'd)	
L-Histidine HCl - H_2O	20.00
Hydroxy-L-proline	10.00
L-Isoleucine	20.00
L-Leucine	60.00
L-Lysine - HCl	70.00
L-Methionine	15.00
L-Phenylalanine	25.00
L-Proline	40.00
L-Serine	25.00
L-Threonine	30.00
L-Tryptophan	10.00
L-Trysine	58.00
L-Valine	25.00
L-Cystaine	260.00
VITAMINS:	
Ascorbic Acid	50.00
D-Biotin	0.01
D-Ca-Pantothanate	0.01
Cholesterol	0.20
Choline Chloride	0.50
Folic Acid	0.01
Inositol	0.05
Niacin	0.025
Niacinamide	0.025
Para-aminobenzoic Acid	0.05
Pyridoxal HCl	0.025
Pyridoxine HCl	0.025
Riboflavin	0.01
Thiamine HCl	0.01

Islet number and viability were serially assessed by dithizone and tripan blue staining. The viability of the islets was further confirmed by dynamic perifusion assays. For this experiment, pancreata was obtained from nine (9) human cadaveric donors.

60 to 80 islets per ml were cultured in either the control medium (CMRL-1066 supplemented with 5 to 10% fetal calf serum and 25 mmol HEPES) or the experimental solution which was the control plus sodium pyruvate at 4 to 15 mmol. The viability and *in vitro* functions of the control and experimental cultures were determined on days 1, 7, 14, 30, 60 and greater than 60 days post isolation.

Table 1 sets out the percent viability on days 7, 14, 30, 60 and greater than 60 days for the experimental and control solutions. The control solution was not able to maintain the cultured islets beyond day 30. On the contrary, greater than 67% of viable islets could be recovered after 60 days of culture in the pyruvate supplemented medium. On 120 days post-isolation, the yield of viable islets was reduced to about 56% for the experimental solution. This is a highly unexpected result in view of the present technology.

The viability of the islet cells was also evaluated *in vivo*. The ability of the cultured islets on days 30, 60 and greater than 60 days to reverse experimentally induced diabetes in a male nude mice model was utilized. The animals were rendered diabetic by a single injection of streptozotocin (185 ml/kg). 72 hours subsequent to the streptozotocin injection, approximately 700 islet cells were implanted into the mice from the experimental and control cultures. The implantation of the islets was under the renal capsule and their function was monitored by serial determinations of blood glucose levels and body weight. The response of the transplanted islets to stress was evaluated on post-operative day 20 by an intraperitoneal glucose tolerance test

(IPGTT). The viability of the transplanted cells was also determined on post-operative day 37 by nephrectomy of the graph bearing kidney.

Islets recovered after 30, 60 and 120 days in the experimental solution culture were transplanted into nude mice and were able to reverse experimentally induced diabetes. Excision of the graph bearing kidney precipitated hyperglycemia in all of the animals which is evidence that euglycemia in these animals was maintained by the transplanted islets.

These results indicate that a culture of islets in a pyruvate-rich medium preserve their long term viability and function. Further, these results support the conclusion that pyruvate containing transplant solutions present an alternative to existing *in vitro* culture and cryopreservation techniques which have yielded variable results to date. Further, the transplant solutions of the present invention allow for the collection of islets from multiple donors, thus optimizing the transplantation of a critical islet cell mass for successful reversal of insulin dependent diabetes mellitus. This data supports the inventive use of pyruvate in transplant solutions. The activity of the pyruvate containing transplant solution or culture medium is in great contrast to the control solution wherein after 1 month, all of the islet cells had lost their capacity to reverse hyperglycemia.

TABLE 1

Viability of Human Pancreatic Islets

Days in Culture	% Viable Experimental (Pyruvate at 5-7 mM)	% Viable Control
7	82	75
14	75	71
30	74	28
60	70	0
60	67	0

EXAMPLE II

Liver Transplant

At present, the use of currently available solutions for the preservation of livers prior to transplantation limits their hypothermic preservation to about 20 hours. There is therefore, a need to develop improved cold-storage solutions. In this Example, a transplant solution in accordance with the present invention was tested against a standard solution presently used by the medical community.

The control solution was lactated Ringer's solution and the experimental solution was the same lactated Ringer's solution containing sodium pyruvate at a 5 mM level.

Donor livers from Lewis rats were orthotopically transplanted into syngeneic rat recipients. The transplantation of the liver occurred after 20 hours of hypothermic (4°C) preservation. Group A, which consisted of 5 rats, had the livers preserved and flushed before revascularization with the control solution. Group B consisted of 5 rats and had the livers preserved with the experimental solution but flushed with the control solution prior to reperfusion. Group C consisted of 5 rats and used the experimental solution for both preservation and flushing. Comparative data was obtained from 3 control rats that did not have the operation. This was Group D.

The operations were performed using accepted techniques in the field. 3 hours after reperfusion of the livers, the animals were sacrificed. The damage to the transplanted livers was assessed by histopathological examination and by liver enzyme serum levels.

Group A (control solution for both preservation and flushing) showed massive injury to the liver. Cell necrosis and degeneration of hepatocytes and non-parenchymal cells was scored as severe. A significant reduction in the histopathological changes was seen in Groups B and C (experimental solution). The changes or level of damage was moderate for Group B and mild for Group C. In Group C large areas of the liver sections presented totally normal morphology.

Serum levels of liver enzymes were increased in all groups, but were significantly lower ($p<0.05$) in Group C. The release of liver enzymes into the serum evidences that damage at the cellular level has occurred. The presence of enhanced levels of liver enzymes in Groups B and C support the inventive transplant solutions and the use of those solutions for preservation and perfusion.

This experiment demonstrates that a pyruvate containing transplant solution protects the liver to be transplanted. This experiment also supports the use of pyruvate containing solutions for extended hypothermic preservation (ischemic period), perfusion of the organ (flushing) and during reperfusion (revascularization).

EXAMPLE III

Liver Transplantation

In this experiment, the liver was transplanted orthotopically in a syngeneic rat recipient after 48 hours of cold storage in University of Wisconsin solution without pyruvate (control, n=3) and with pyruvate (control, n=3). The experimental Wisconsin solution contained 5 mM calcium pyruvate. Before revascularization of the liver graft, the vasculature was flushed with Ringer's lactate (control) and Ringer's lactate containing 5 mM pyruvate (study group). In the livers of animals transplanted with organs preserved and flushed with solutions containing pyruvate, was

observed a reduced in parenchymal necrosis, apoptosis of hepatocytes and necrosis of non-parenchyal cells.

The data demonstrate that pyruvate protects the liver to be transplanted after an extended hypothermic preservation time (48 hours), reducing the injury observed when preserved with the best preservation solution available today for human livers and other organs.

EXAMPLE IV

Intestinal Transplant

Interluminal Installation of Pyruvate

This experiment was conducted to demonstrate that insertion of a pyruvate containing diet into the lumen of the small intestine prior to harvesting, results in improved viability of the transplanted organ.

The small intestine of 12 Sprague Dawley rats was tied to closure. The bowel downstream from the closure received directly about 10 cc's of a placebo liquid diet which consisted of a standard liquid rat chow containing polyglucose (n=6) or the same liquid diet with 10% of the energy (caloric) content of the polyglucose being displaced by a mixture of sodium and calcium pyruvate (n=6).

The grafts were then harvested and stored for 2 hours in cold (4°C) Ringer's solution. The grafts were then transplanted with additional insertion of the experimental and control diets into the lumen.

Mucosal parameters were compared between the untreated and pyruvate treated grafts two hours after reperfusion. Tissue injury was evaluated by histopathology. Mucosal injury, after

reperfusion of the preserved grafts and during acute rejection was significantly inhibited by pyruvate.

Pyruvate treatment before cold preservation of intestinal grafts, in this rat model, reduced the amount of damage occasioned upon the graft. After reperfusion of the hypothermically preserved grafts, the separation of the villous epithelium from the lamina propria extended almost the full length of the villi and in some sections, destruction of the villous tips was also observed. In the grafts treated with pyruvate, there was a marked decrease in edema and separation of the epithelium from the lamina propria was not observed.

EXAMPLE V

Intestine Transplantation

This experiment is designed to evaluate the use of pyruvate containing preservation solutions in the transplant of small bowel tissue. Small bowel grafts are harvested from male Sprague Dawley rats and are stored in cold (4°C) Ringer's lactate solution for 2 hours prior to undergoing orthotopic transplantation into Sprague Dawley recipients. The Control group grafts are stored in standard Ringer's solution while the Experimental group grafts are stored in Ringer's solution with sodium pyruvate at 10 mM. Small bowel biopsy specimens are obtained before harvesting, before revascularization and 30, 60, 120 minutes and 24 hours after transplantation.

After reperfusion of the hypothermically preserved grafts, the separation of the villous epithelium from the lamina propria will extend almost the full length of the villi and in some sections, destruction of the villous tips will be observed. In the grafts stored in the pyruvate Ringer's, there is a marked decrease I edema and separation of the epithelium from the lamina

propria. In general, the grafts stored in the pyruvate containing solution survived the hypothermic storage much better than the Controls.

EXAMPLE VI

Feeding Pyruvate Before Transplantation

One aspect of the present invention relates to the discovery that feeding of pyruvate to the donor prior to removal of the organ and feeding of pyruvate to the recipient prior to and subsequent to transplantation can provide a beneficial effect. This experiment used rejection of an intestinal graft in rats to demonstrate the beneficial effects of feeding pyruvate for transplantation procedures.

Small bowel grafts were transplanted across an allogenic (ACI strain of rat to Lewis strain of rat) barrier. Grafts obtained from 4 untreated rats (not fed pyruvate) were used as controls. The experimental group of 4 rats were fed pyruvate at 5 gms per day for 7 days prior to harvesting. The recipients of the experimental group grafts were fed pyruvate at 5 gms per day for 6 days post transplantation. Observations obtained from native intestines serves as a baseline for comparisons.

6 days subsequent to transplantation, the animals were sacrificed and the grafts were assessed for severity of injury by histopathological examination. The examination of the grafts revealed that the experimental group suffered a significant reduction of cellular damage and cell death. There was also a significant reduction in endothelial cell damage, leukocyte adhesion and intravascular platelet aggregation. The experimental group also evidenced preservation of Goblet

cells and increased mitotic events as compared to controls. These observations support the feeding of pyruvate prior to and/or subsequent to transplantation of organs.

Industrial Applicability

The medical community is constantly searching for improvements to the transplantation of organs. The present invention provides a transplant solution of improved performance and a method for the transplantation of organs that increases the likelihood of a successful clinical outcome. The transplant solutions of this invention are isotonic aqueous solutions that contain from 1 to 100 mM, more preferably from 1 to 25 mM of pyruvate. These isotonic solutions may also contain antibiotics, anti-inflammatories and other components known in the art. An additional aspect of the invention is a method for organ transplantation that comprises feeding the organ recipient pyruvate prior to and/or subsequent to the transplant operation.

While the transplant solutions and the method for organ transplant of this invention have been described herein and constitute preferred embodiments of the invention, it is to be understood that the invention is not limited to these precise solutions or methods and that changes may be made therein without departing from the scope of the invention, which is defined in the appended claims.

What is claimed is:

1. A transplant solution comprising water, a buffer system and pyruvate.

2. The transplant solution according to Claim 1 wherein the pyruvate is selected from the group consisting of sodium pyruvate, calcium pyruvate, potassium pyruvate and mixtures thereof.

3. The transplant solution according to Claim 1 wherein the pyruvate is at a concentration of 1-100 mM.

4. The transplant solution according to Claim 1 wherein the pyruvate is at a concentration of 2 to 25 mM.

5. A transplant solution according to Claim 1 further comprising at least one element selected from the group consisting of antibiotics, steroids, insulin, nucleotides, carbohydrates and peptides.

6. A method for the transplantation of an organ in a mammal, said method comprises the administration of pyruvate to the recipient animal.

7. The method according to Claim 6 wherein the administration of the pyruvate is enteral administration.

8. The method according to Claim 6 wherein the administration of the pyruvate is parenteral administration.

9. The method according to Claim 6 wherein the administration of the pyruvate is prior to the transplantation of the organ.

10. The method according to claim 6 wherein the administration of the pyruvate is subsequent to the transplantation of the organ.

11. A method for the transplantation of an organ in a mammal, said method comprises the perfusion of the organ with a transplant solution comprising water, a buffer system and pyruvate prior to the establishment of the blood flow from the recipient mammal to the organ.

12. The method according to Claim 11 wherein said pyruvate is at a concentration of 1-100 mM and wherein said transplant solution further comprises at least one element selected from the group consisting of antibiotics, steroids, insulin, nucleotides, carbohydrates and peptides.

13. A transplant solution comprising water, at least one steroid at a concentration of from 2 -20 mg/L, at least one carbohydrate at a concentration of from 10 to 100 mmol at least one nucleoside at a concentration of 1-20 mmol, at least one antibiotic at a concentration of 0.1 to 1.0 mg/L and pyruvate at a concentration of 1 to 100 mmol and wherein said transplant solution has an Osmolality of 250-375 mmos.

14. The transplant solution according to Claim 13 wherein said pyruvate is at a concentration of 2 to 25 mM and is selected from the group consisting of sodium pyruvate, calcium pyruvate, magnesium pyruvate, potassium pyruvate and mixtures thereof.

15. The transplant solution according to Claim 13 wherein the pyruvate is at a concentration of 2-15 mM and wherein the transplant solution has an Osmolality of 275-350 mmos.

16. The transplant solution according to Claim 13 wherein the pyruvate is selected from the group consisting of sodium pyruvate, and pyruvate precursors.

17. The transplant solution according to Claim 13 further comprising steroids, insulin and peptides.

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(54) Title: TRANSPLANT SOLUTIONS CONTAINING PYRUVATE AND METHODS FOR TRANSPLANTATION

(57) Abstract

The present invention is based upon the discovery that the use of pyruvate in transplant solutions and the feeding of pyruvate and/or pyruvate derivatives to the organ recipients prior to and subsequent to transplantation, greatly enhances the survivability of the recipient and the viability of the transplanted organ. According to the present invention, providing pyruvate prior to the transplantation of an organ and/or subsequent to the transplantation of the organ, will significantly increase the likelihood of a successful transplantation. Further, the use of pyruvate-containing preservation, transplant, perfusion and pyruvate irrigation solutions will greatly enhance the maintenance of the organs prior to transplantation. The transplant and irrigation solutions of the present invention contain components that are known in the art and pyruvate at a concentration from 1 to 100 mM.

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INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 97/13160

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A01N1/02 A61K31/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 536 751 A (R. BUNGER) 16 July 1996 see column 7, line 47 - line 53 see column 8, line 5 see column 8, line 41 - line 44 see column 19, line 19 - line 41 see examples	1-17
X	WO 91 09520 A (UNIV CALIFORNIA) 11 July 1991 see claims	1-17
X	WO 92 04826 A (UNIV MINNESOTA) 2 April 1992 see claims see page 3, line 20 - line 28	1-17
X	EP 0 215 138 A (NESTLE SA) 25 March 1987 see claims	1-17

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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search	Date of mailing of the international search report
3 December 1997	15.01.98

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 9205 Derwent Publications Ltd., London, GB; Class B05, AN 92-039875 XP002049017 & SU 1 632 427 A (UNIV KHARK) , 7 March 1991 see abstract	1-17
X,P	US 5 599 659 A (BRASILE LAUREN ET AL) 4 February 1997 see claims	1-17
X	US 5 294 641 A (STANKO RONALD T) 15 March 1994 cited in the application see column 3, line 24 - line 38	6-10
X	BIOLOGICAL ABSTRACTS, vol. 96, 1993 Philadelphia, PA, US; abstract no. 37299, S. KOJIMA ET AL.: "Simple 24-hour preservation of rabit hearts with hexanol and pyruvate cardioplegia." XP002049016 see abstract & J. MOL. CELL CARDIOL., vol. 25, no. 4, 1993, pages 453-458,	1-17

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/13160

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 6-10 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/US 97/13160

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9109520 A	11-07-91	US 5066578 A	19-11-91
		US 5075210 A	24-12-91
		AU 7175591 A	24-07-91
WO 9204826 A	02-04-92	AU 8539491 A	15-04-92
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US 5294641 A	15-03-94	WO 9511018 A	27-04-95
		EP 0680317 A	08-11-95
		JP 9500144 T	07-01-97

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